Distribution of thiabendazole and thiophanate-methyl resistant strains of *Helminthosporium solani* and *Fusarium sambucinum* in Alberta potato storages

J.D. Holley[1] *and L.M. Kawchuk*[2]

Thiabendazole resistant strains of *Helminthosporium solani*, the silver scurf pathogen, were recovered from 15 of 32 farms surveyed in 1994. Resistance was primarily limited to southern Alberta where 76% of the farms were affected. Only 21% of the farms in the north had a problem. Resistant isolates of Fusarium sambucinum, one of the dry rot pathogens, were found on 12 of 31 farms. In this case, thiabendazole resistance was found on 67% of the farms surveyed in southern Alberta. There was no evidence of resistance in any storage facility in the north. Thiabendazole resistant isolates of both pathogens were invariably cross-resistant to thiophanate-methyl.

Can. Plant Disease Surv. 76:1, 21-27, 1996.

Des souches de *Helminthosporium solani*, la tache argentée, devenues résistantes au thiabendazole ont été retrouvées dans 15 fermes sur 32 en 1994. La résistance était limitée en premier lieu au sud de l'Alberta, là où 76 % des fermes étaient touchées par la maladie. Seulement 21 % des fermes dans le nord avaient un problème. Des isolats résistants au Fusarium sambucinum, un des pathogènes de la pourriture sèche, ont été retrouvés dans 12 des 31 fermes. Pour ce qui est de çette maladie, 67 % des fermes examinées dans le sud de l'Alberta ont démontré une résistance au thiabendazole. Aucun cas de résistance n'a été décelé dans les entrepôts du nord. Les isolats résistants au thiabendazole des deux pathogènes présentaient de façon invariable une résistance croisée au thiophanate-méthyl.

Introduction

Levels of silver scurf, caused by *Helminthosporium solani*, began to rise in many storages in Alberta in the mid 1980's. Chips made from potatoes with severe infection had so many black burnt edges that packaged product could not be sold. By 1993, skin freckling from silver scurf was so severe that some Alberta fresh market potatoes were rejected by retail grocery chains. In the early 1990's, abnormally high levels of dry rot decay, caused by *Fusarium sambucinum* and four other fusarium species, developed unexpectedly in a few potato storages.

High levels of both diseases were observed even where registered seed piece treatments or postharvest fungicides had been applied. Thiabendazole resistance had been found earlier in potato storages for both pathogens (1,2,4,5). Thiophanate-methyl (Easout), a fungicide that is registered as a seed piece treatment for silver scurf, is very similar chemically to thiabendazole. Results showed that both pathogens had developed resistance to both chemicals (3). A relatively small number of isolates of *H. solani* and *F. sambucinum* were tested for sensitivity to these fungicides between 1990 and 1993. Although the results showed that each pathogen could be resistant to both fungicides (3), they did not clearly show how common resistant strains were within each storage or how widespread the problem had become in Alberta. This information is very important in deciding whether using these fungicides is still advisable. Results from a more comprehensive survey, which demonstrates the incidence of fungicide resistance in Alberta potato storages in 1995, are presented in this report.

Materials and methods

Potatoes with symptoms of silver scurf and dry rot were obtained from 41 farms from every growing region in Alberta. The tubers were washed, examined closely for symptoms, and a number assigned to identify its source.

Tubers with silver scurf were wrapped in wet paper towels, sealed in plastic bags and incubated for 4-7 weeks at 20°C. Individual *H. solani* spores were removed with a fine needle from skin surfaces and transferred onto acidified potato dextrose agar (PDA-A) or potato dextrose agar (PDA) amended with penicillin and streptomycin (PDA-PT). Colonies with *H. solani* spores were subcultured onto malt agar (MA) and incubated for 5-8 weeks at 20°C. Sensitivity to thiabendazole was determined for 84 pure cultures.

1 Alberta Agriculture Food and Rural Development, Research Centre, S.S. #4, Brooks, Alberta, Canada T1R 1E6.
2 Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1.
Accepted for publication, January 12, 1996.

Tubers with cuts or pressure bruises were sliced open and wefts of mycelia inside dry rot cavities placed on PDA-PT to isolate fusarium dry rot fungi. If mycelia were not present, tissue was removed from edges of dry rot lesions, surface sterilized and plated onto PDA-A or PDA-PT. Colonies with macroconidia were transferred to fresh PDA and incubated for 7-14 days at 20°C. The fusarium cultures were subjected to a pre-test. Small round plugs of densely colonized media, 5 mm in diameter, were removed from the edge of each culture and plated onto PDA amended with 0, 5 or 10 mg/L of thiabendazole. Colonies that grew uninhibited were purified using a standard single spore technique (6) and then tested against a full range of fungicide concentrations. Several isolates from each farm that were inhibited by low concentrations of thiabendazole in the pre-test were also single spored.

Plugs 5 mm in diameter were removed from pure colonies of *H. solani* and from single spore cultures of fusarium. Plugs of *H. solani* were transferred onto MA plates amended with 0, 0.05, 0.5, 5, 50, 200 and 500 mg/L of thiabendazole or thiophanate-methyl. Plugs of fusarium were transferred onto PDA plates amended similarly. Two plates of each test concentration were inoculated with plugs. Radial diameters of all *H. solani* isolates were measured 60 days after inoculation even when colonies on unamended MA had not grown out to the edges of the plates. Radial diameters of all fusarium colonies were measured when colonies on unamended PDA reached the edges of the plates, usually after 7 to 35 days of incubation depending on the species.

Colony diameters of each isolate were regressed against natural logarithms of matching test concentrations to determine its response to the fungicides. Equations derived from these regressions were used to determine the concentration of each fungicide that was required to inhibit the radial diameter of each colony by 50%. This concentration or EC_{50} value was then used to represent the sensitivity of each isolate to each fungicide.

Results

The radial growth of 46 (55%) of 84 *H. solani* isolates tested was only marginally suppressed by moderately high (60 to 150 mg/L) to very high (µ500 mg/L) levels of thiabendazole (Table 1, 2). Very resistant isolates had EC_{50} values that ranged from 150 to over 500 mg/L. These isolates were found in the storage facilities of 13 (76%) of 17 farms in southern Alberta (Table 1) and also in storages of 3 (21%) of 14 farms in the north (Table 2). Some isolates were resistant, i.e. had EC_{50} values from 60 - 150 mg/L. These isolates were found on 5 (36%) of 17 farms in the south. The remaining isolates were either sensitive or very sensitive with EC_{50} values from 0.1 to 15 mg/L. They came from 5 (36%) of 17 farms in the south and from 12 (86%) of 14 farms in the north. It was quite uncommon to recover both sensitive and resistant isolates from the same farm. Isolates that tolerated even moderately high concentrations of thiabendazole grew uninhibited on very high concentrations of thiophanate-methyl (Table 3). Isolates that were sensitive to thiabendazole were also sensitive to thiophanate-methyl.

A total of 32 (43%) of 75 *F. sambucinum* isolates recovered in the 1994 survey showed resistant responses to thiabendazole (Table 4,5). The growth of the other fusarium species was inhibited by low concentrations of the fungicide. Resistant *F. sambucinum* isolates were recovered from 12 (67%) of 18 farms sampled in southern Alberta (Table 4). There was no evidence of resistance in the north (Table 5). Ten very resistant isolates, i.e. isolates with EC_{50} values greater than 125 mg/L, were recovered from 5 (28%) of 18 farms surveyed in the south. The other 22 resistant isolates, i.e. those with EC_{50} values above 20 but below 125 mg/L, were from 11 (61%) of these same farms. The remaining 43 sensitive isolates had EC_{50} values from 2-10 mg/L and came either from storages in northern Alberta or from 2 farms in the south. Although it was rare to recover sensitive and resistant isolates of *F. sambucinum* from the same farm, it was common to isolate other sensitive fusarium dry rot pathogens from farms that had a problem. Each thiabendazole resistant *F. sambucinum* isolate was also resistant to thiophanate-methyl (Table 6). Isolates that were sensitive to one fungicide were also sensitive to the other.

Discussion

Thiabendazole and thiophanate-methyl are the only fungicides specifically registered for dry rot and silver scurf. Other fungicides are registered for seed piece decay, however, it is not clear from their labels whether they would be effective against dry rot and silver scurf in storage. Loss of efficacy of thiabendazole and thiophanate-methyl could leave potato growers with no chemical controls for these two storage diseases (7). Costs of registering a chemical for use on a food product are so prohibitive that chemical companies have not shown any interest in sponsoring the application for new fungicide labels.

It is not clear at this time how long these two fungicides will continue to be effective. Resistant strains were not recovered or were rare on many farms in Alberta, particularly in the north (Tables 1 and 3). Low levels of sensitive isolates of *H. solani* in some storages facilities (Table 1), and the dramatic rise in the incidence and severity of silver scurf suggests that a significant population shift and subsequent loss of fungicide efficacy may have occurred for the silver scurf pathogen on many farms in southern Alberta. In contrast to this, thiabendazole sensitive dry rot isolates were found in almost every storage sampled (Table 3). Extensive dry rot decay is still relatively uncommon in Alberta. These findings indicate that the thiabendazole and thiophanate-methyl may still effectively control dry rot in many storages

Table 1. Concentrations of thiabendazole required to inhibit the growth of *Helminthosporium solani* isolates recovered from storages in southern Alberta by 50% measured in milligrams per litre (mg/L).

Farm Number	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
1	2	≥500	Very resistant
2	2	385 - ≥500	Very resistant
	1	145	Resistant
3	4	2 - 4	Sensitive
4	1	≥500	Very resistant
	1	125	Resistant
5	9	200 - ≥500	Very resistant
6	2	265 - ≥500	Very resistant
	1	2	Sensitive
7	3	2 - 9	Sensitive
8	1	≥500	Very resistant
9	2	175 - ≥500	Very resistant
	1	100	Resistant
10	2	2 - 3	Sensitive
11	2	150 - ≥500	Very resistant
	1	60	Resistant
12	3	≥500	Very resistant
	1	135	Resistant
13	3	210 - ≤500	Very resistant
	1	125	Resistant
14	3	≥500	Very resistant
15	3	150 - ≥500	Very resistant
	1	125	Resistant
16	1	≥500	Very resistant
17	2	1 - 7	Sensitive

for some time to come, if they are applied using sprayers that are calibrated properly at the full label rate (8) and if they are applied only when they are really needed.

In conclusion, results from the 1994 survey indicate that there is an urgent need to develop a more integrated approach for controlling silver scurf and dry rot in storage, i.e. one that reduces the reliance on chemicals (7). Reducing the use of these fungicides is critical to preserving their efficacy. This is very important since it seems unlikely that there will be a new fungicide to replace them in the near future.

Acknowledgments

Funding for this research was provided by the Alberta Potato Research Association, a grower sponsored organization, and by the Alberta Agricultural Research Institute's Matching Grants Program, a program supported by the province of Alberta. The authors would like to thank Sherry Lisowski and Debbie Topinka for isolating, identifying, and testing the plant pathogens.

Literature cited

1. Hide, G.A., Hall, S.M. and Boorer, K.J. 1988. Resistance to thiabendazole in isolates of *Helminthosporium solani,* the cause of silver scurf disease of potatoes. Plant Pathol. 37:377-380.
2. Hide, G.A., Read, P.J. and Hall, S.M. 1992. Resistance to thiabendazole in Fusarium species isolated from potato tubers affected by dry rot. Plant Pathol. 41:745-748.
3. Kawchuk, L.M., Holley, J.D., Lynch, D.R. and Clear, R.M. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani.* Am. Potato J. 71:185-192.
4. Langerfield, E. 1986. Thiabendazol-resistenz bei *Fusarium sulphureum.* Nachrichtenbl Deut Pflanzenschutzd 38:165-168.
5. Merida, C.L. and Loria, R. 1990. First report of resistance of *Helminthosporium solani* to thiabendazole in the United States. Plant Dis. 74: 614.
6. Nelson, P.E., Toussoum, T.A. and Marasas, W.F.O. 1983. Fusarium species. An illustrated manual for identification. Pennsylvania State University Press. 193p.
7. Olivier, C. and Loria, R. 1995. Alternative fungicides for postharvest control of silver scurf on potato tubers. Phytopathology 85:1126.
8. Szeto, S.Y., Joshi, V., Price, M.P. and Holley, J. 1993. Persistence and efficacy of thiabendazole on potatoes for control of silver scurf. J. Agric. Food Chem. 41:2156-2159.

Table 2. Concentrations of thiabendazole required to inhibit the growth of *Helminthosporium solani* isolates recovered from storages in northern Alberta by 50% measured in milligrams per litre (mg/L).

Farm Number	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
18	3	2 - 10	Sensitive
19	4	2 - 6	Sensitive
20	2	330 - ≥500	Very resistant
21	2	2 - 3	Sensitive
22	4	3 - 7	Sensitive
	1	0.1	Very sensitive
23	3	2 - 6	Sensitive
24	3	7 - 15	Sensitive
25	2	2 - 3	Sensitive
26	1	10	Sensitive
27	2	2 - 12	Sensitive
28	1	250	Very resistant
29	1	4	Sensitive
30	1	2	Sensitive
31	1	150	Very resistant

Table 3. Concentrations of thiophanate-methyl required to inhibit the growth of *Helminthosporium solani* isolates by 50% measured in milligrams per litre (mg/L).

Farm Number	Region	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
1	South	1	≥500	Very resistant*
2	South	1	≥500	Very resistant
3	South	1	1	Sensitive
4	South	1	≥500	Very resistant
5	South	1	≥500	Very resistant
6	South	1	≥500	Very resistant
		1	1	Sensitive
7	South	1	7	Sensitive
9	South	1	≥500	Very resistant
10	South	1	1	Sensitive
11	South	1	≥500	Very resistant
13	South	1	≥500	Very resistant
14	South	1	≥500	Very resistant
15	South	1	≥500	Very resistant
17	South	1	2	Sensitive
18	North	1	4	Sensitive
19	North	1	4	Sensitive
20	North	1	≥500	Very resistant
21	North	1	1	Sensitive
22	North	1	5	Sensitive
23	North	1	1	Sensitive
24	North	1	1	Sensitive
25	North	1	0.05	Very sensitive
26	North	1	5	Sensitive
27	North	1	0.1	Very sensitive
28	North	1	≥500	Very resistant

* Isolates that were very resistant to thiophanate-methyl were either resistant or very resistant to thiabendazole. Isolates sensitive to thiophanate-methyl were also sensitive to thiabendazole.

Table 4. Concentrations of thiabendazole required to inhibit the growth of *Fusarium sambucinum* and other fusarium dry rot fungi recovered from storages in southern Alberta by 50% measured in milligrams per litre (mg/L).

Farm Number	Fusarium Dry Rot Species Isolated	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
1	*F. sambucinum*	4	≥125	Very resistant
	Other species	4	3 - 8	Sensitive
2	*F. sambucinum*	2	≥125	Very resistant
	F. sambucinum	1	105	Resistant
	Other species	1	2	Sensitive
3	*F. sambucinum*	2	2	Sensitive
	Other species	10	3 - 5	Sensitive
4	*F. sambucinum*	1	≥125	Very resistant
	F. sambucinum	4	40 - 125	Resistant
	Other species	1	4	Sensitive
5	*F. sambucinum*	1	≥125	Very resistant
	F. sambucinum	2	37 - 40	Resistant
	Other species	2	4 - 10	Sensitive
6	*F. sambucinum*	2	≥125	Very resistant
	F. sambucinum	2	60 - 120	Resistant
	Other species	2	1	Sensitive
	Other species	1	0.1	Very sensitive
7	*F. sambucinum*	2	60 - 70	Resistant
8	Other species	1	5	Sensitive
9	*F. sambucinum*	1	120	Resistant
	Other species	2	2	Sensitive
10	*F. sambucinum*	1	110	Resistant
	F. sambucinum	2	2	Sensitive
	Other species	2	2	Sensitive
11	*F. sambucinum*	2	45 - 100	Resistant
	Other species	1	2	Sensitive
12	*F. sambucinum*	2	40	Resistant
13	*F. sambucinum*	1	70	Resistant
14	Other species	4	1 - 5	Sensitive
	Other species	2	0.1	Very sensitive
15	Other species	2	0.01	Very sensitive
16	*F. sambucinum*	3	23 - 45	Resistant
17	*F. sambucinum*	2	38 - 39	Resistant
	Other species	1	5	Sensitive
18	Other species	1	3	Sensitive

Table 5. Concentrations of thiabendazole required to inhibit the growth of *Fusarium sambucinum* and other fusarium dry rot fungi recovered from storages in northern Alberta by 50% measured in milligrams per litre (mg/L).

Farm Number	Fusarium Dry Rot Species Isolated	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
19	*F. sambucinum*	2	2	Sensitive
	Other species	1	5	Sensitive
20	*F. sambucinum*	5	2	Sensitive
	Other species	4	3 - 8	Sensitive
21	*F. sambucinum*	1	2	Sensitive
	Other species	6	2 - 6	Sensitive
22	*F. sambucinum*	4	2 - 4	Sensitive
	Other species	13	2 - 5	Sensitive
23	*F. sambucinum*	2	4 - 5	Sensitive
	Other species	5	3 - 8	Sensitive
24	Other species	3	4 - 5	Sensitive
25	*F. sambucinum*	4	2 - 10	Sensitive
	Other species	7	4 - 9	Sensitive
	Other species	1	0.01	Very sensitive
26	*F. sambucinum*	1	2	Sensitive
	Other species	6	2 - 12	Sensitive
27	Other species	5	2 - 5	Sensitive
	Other species	1	0.01	Very sensitive
28	*F. sambucinum*	5	2 - 6	Sensitive
	Other species	8	2 - 5	Sensitive
29	*F. sambucinum*	8	4 - 8	Sensitive
	Other species	12	3 - 13	Sensitive
	Other species	2	0.01	Very sensitive
30	Other species	5	2 - 10	Sensitive
	Other species	1	0.1	Very sensitive
31	*F. sambucinum*	7	1 - 2	Sensitive
	Other species	4	4 - 7	Sensitive

Table 6. Concentrations of thiophanate-methyl required to inhibit the growth of *Fusarium sambucinum* and other fusarium dry rot fungi recovered from storages all across Alberta by 50% measured in milligrams per litre (mg/L).

Farm Number	Region	Fusarium Dry rot Species Isolated	Number of Isolates Tested	Range of EC_{50} Values (mg/L)	Isolate Sensitivity
1	South	*F. sambucinum*	2	≥150	Very resistant*
2	South	*F. sambucinum*	2	150	Very resistant
4	South	*F. sambucinum*	1	≥150	Very resistant
5	South	*F. sambucinum*	1	≥150	Very resistant
6	South	*F. sambucinum*	2	≥150	Very resistant
9	South	*F. sambucinum*	1	≥150	Very resistant
10	South	*F. sambucinum*	1	≥150	Very resistant
		F. sambucinum	1	2	Sensitive
11	South	*F. sambucinum*	1	≥150	Very resistant
13	South	*F. sambucinum*	1	≥150	Very resistant
31	North	*F. sambucinum*	3	3	Sensitive

* Isolates that were very resistant to thiophanate-methyl were either resistant or very resistant to thiabendazole. Isolates sensitive to thiophanate-methyl were also sensitive to thiabendazole.